

Fig. 1

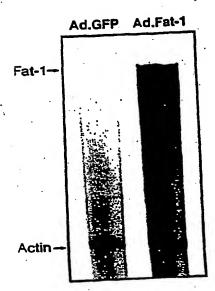
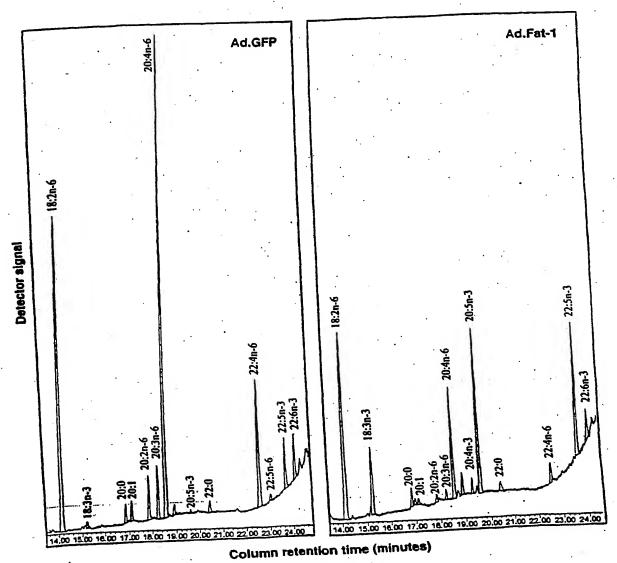


Fig. 2



WIMINI TOTAL

Fig. 3

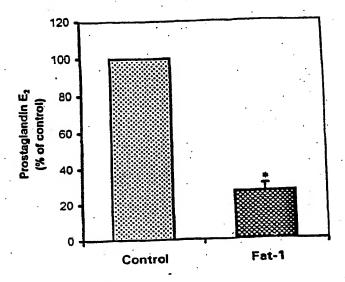


Fig. 4

	-	
Mol % of total Fatty acids	Control	Fat-1
n-6 Polyunsaturate	S	- b .
18:2n-6	14.2°	9.2 ^b
20:2n-6	1.2° .	0.3 ^b
20:3n-6	1.6ª	0.4 ^b
20:4n-6	15.2°	4.1 ^b
22:4n-6	4.4 ⁸	1.0 ^b
22:5n-6	0.2ª	0.0 ^b
Total	36.8ª	15.0 ^b
n-3 Polyunsaturāte	es ·	
18:3n-3	0.2	3.6ª
20:4n-3	0.0 ^b	0.6
20:5n-3	0.1 ^b	6.1 ³
22:5n-3	1.2 ^b	5.8ª
22:6n-3	1.0ª	1.3 ^a
•	2.5 ^b	17.4°
Total		
n-6/n-3 Ratio	14.7	0.9 ^b
11 2000 2 0000		1 Maliana for

Values are means of four measurements. Values for each fatty acid with the same letter do not differ significantly (P<0.01) between control and fat-1.

Fig. 5

Assessment of expression of the transgene of the transgene of the n-3 desaturase

B

Construction of viral expression vector

Cell culture

Assessment of expression of the pression of the transgene of the n-3 desaturase

Examination of activity of the n-3 desaturase

B

Examination of activity of the n-3 desaturase

Cell culture

Examination of activity of the n-3 desaturase

Cell culture

Examination of activity of the n-3 desaturase

Cell culture

Cell culture

Examination of activity of the n-3 desaturase

Cell culture

Cell culture

Calculate of the n-3 desaturase

Cell culture

Cell culture

Cell culture

Cell culture

Assessment of expression of the n-3 desaturase

Cell culture

Cell cul

diet for Gene transfer Organ harvest at day 2, 4, 10, 30 & 60 after gene transfer (Viral delivery) 30 & 60 after gene transfer

8

Ad.GFP (8 rats x 5) (8 rats/per time point)
Gene transcripts (mRNA)
Fatty acid composition
Eicosanoid contents

gh n-3 FA diet (no gene transfer, as endpoint reference)

vector onstruction

AG + Fat-l

 Tissue harvest → Biochemical analyses

Gene transcripts (mRNA Fatty acid composition Eicosanoid contents

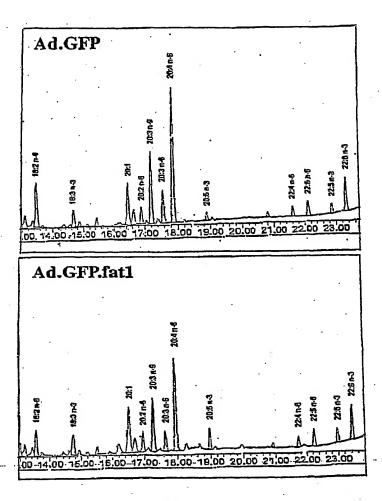


Fig. 9

PUFA composition of total cellular lipids from the control rat cortical and the transgenic cells expressing a C. elegans fat-1 cDNA

Mol % of total fatty acids	Control	fat-1	
n-6 Polyunsaturates			
18:2n-6	1.78	0.87	
20:4n-6	7.21	4.23	
22:4n-6	1.57	0.72	•
22:5n-6	1.68	0.72	
Total	12.26	6.53	
n-3 Polyunsaturates		8	-
18:3 u- 3	0.34	0.86	
20:5n-3	0.21	0.87	
22:5n-3	0.29	0.81	
22:6n-3	1.27	1.93	
Total	2.11	4.48	
n-6/n-3 Ratio	6.44	1.67	

Values are means of four measurements. (p<0.01) between control and fat-1.

Fig. 10

Prostaglandin E2

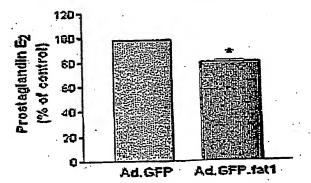


Fig. 11

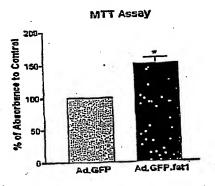


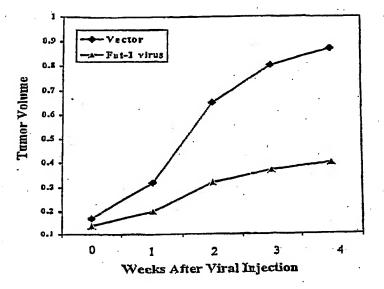
Fig. R

Ad.GFP



Ad.GFP.Fat-1





Fg . 14

PUFA composition of total cellular lipids from the control MCF-7 and the transgenic cells expressing a C. elegans fat-1 cDNA

Mol % of total fatty acids	Control	Fat-1			
n-6 Polyunsaturates					
18:2n-6 20:2n-6 20:3n-6 20:4n-6 22:4n-6 22:5n-6 Total	3.13° 0.23° 0.34° 6.30° 0.53° 0.27° 10.80°	1.51 ^b 0.22 ^a 0.16 ^b 2.26 ^b 0.33 ^b 0.11 ^b 4.59 ^b			
n-3 Polyunsaturates					
18:3n-3 20:4n-3 20:5n-3 22:5n-3 20:5n-3 Total	0.0 ^b 0.0 ^b 0.33 ^b 0.60° 0.93 ^b	1.00° 0.10° 2.87° 1.47° 0.73° 6.17°			
n-6/n-3 Ratio	11.61ª	0.74 ^b			

Values are means of four measurements. Values for each fatty acid with the same letter do not differ significantly (p<0.01) between control and fat-1.

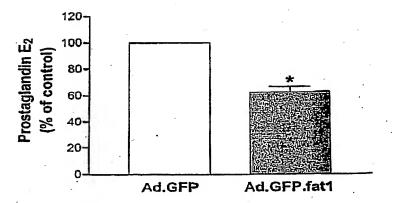


Fig. 16

an a compact C CT	
CAAGTITGAG GT	· 57
ATG GTC GCT CAT TCC TCA SAA GGG TTA TCC GCC ACG GCT CCG GTC	31
Met Val Ala His Ser Ser Glu Gly Leu Ser Ala Thr Ala Pro Val	,
*	102
ACC GGC GGA GAT GTT CTG GTT GAT GCT CGT GCA TCT CTT GAA GAA	102
Thr Gly Gly Asp Val Leu Val Asp Ara Arg Ara Ser Hea Cla 30	
20	- 47
AAG GAG GCT CCA CGT GAT GTG AAT GCA AAC ACT AAA CAG GCC ACC	147
Lys Glu Ala Pro Arg Asp Val Ash Ala Ash Int 270 011 45	e
ACT GAA GAG CCA CGC ATC CAA TTA CCA ACT GTG GAT GCT TTC CGT	192
Thr Glu Glu Pro Arg Ile Gln Leu Pro Illi vel Asp 122 60	
50	
CGT GCA ATT CCA GCA CAC TGT TTC GAR AGA GAT CTC GTT AAA TCA	237
Arg Ala Ile Pro Ala His Cys and Git Ala Asp 25 75	
65	202
ATC AGA TAT TTG CTG CAA GAC TTT GCG GCA CTC ACA ATT CTC TAC	282
Ile Arg Tyr Leu Val Gin Asp Phe Ala ala 200 90	
. 60	327
TTT GCT CTT CCA GCT TTT GAG TAC TTT GGA TTG TTT GGT TAC TTG	321
Phe Ala Leu Pro Ala Phe Giu Tyr Phe Giy 100	
95 FOO PMC STC	372
GTT TGG AAC ATT TTT ATG GGA GTT TTT GGA TTC GCG TTG TTC GTC Val Trp Asn Ile Phe Met Gly Val Phe Gly Phe Ala Leu Phe Val	
Val Trp Asn Ile Phe Met Gly Val Phe Gly 115	
·	417
GTT GGA CAC GAT TGT CTT CAT GGA TCA TTC TCT GAT AAT CAG AAT Val Gly His Asp Cys Leu His Gly Ser Phe Ser Asp Asn Gln Asn 135	
Val Gly His Asp Cys Lett His Gly 555 130	
	462
CTC AAT GAT TTC ATT GGA CAT ATC GCC TTC TCA CCA CTC TTC TCT Leu Asn Asp Phe lie Gly His Ile Ala Phe Ser Pro Leu Pho Ser 150	
145 150	
CCA TAC TTC CCA TGG CAG AAA AGT CAC AAG CTT CAC CAT GCT TTC	507
CCA TAC TTC CCA TGG CAG AAA AGT CAC Lys Leu His His Ala Phe Pro Tyr Phe Pro Trp Gln Lys Ser His Lys Leu His His Ala Phe	
155 160	7
ACC AAC CAC ATT GAC AAA GAT CAT GGA CAC GTG TGG ATT CAG GAT	552
The Asp His Ile Asp Lys Asp Lis Gif))
170	
AAG GAT TGG GAA GCA ATG CCA TCA TGG AAA AGA TGG TTC AAT CC	A 597
The Asp Trp Glu Ala Met Pro Ser 119 130 119 119	0. 5
185	•
ATT CCA TTC TCT GGA TGG CTT AAA TGG TTC CCA GTG TAC ACT TT	A .642
The Pro Phe Ser Gly lip her mys inprove	0
200	
TTC GGI TTC TGI GAT GGA TCT CAC TTC TGG CCA TAC TCT TCA CI	rg 687
THE GGI TIC 191 ONE THE	

Fig. 17 A

Phe	Gly	Phe	Cys	Asp 215	Gly	Ser	His	Phe	Trp 220	Pro	Tyr	Ser	Ser	Leu 225	. •
TTT Phe	GTT Val	CGT Arg	AAC Asn	TCT Ser 230	GAC Asp	CGT Arg	GTT Val	CAA Gln	TGT Cys 235	GTA Val	ATC Ile	TCT Ser	GTA	ATC 11e · 240	732
TGT Cys	TGC Cys	TGT Cys	GTG Val	TGT Cys 245	GCA Ala	TAT Tyr	ATT	GCT Ala	CTA Leu 250	ACA Thr	ATT	GCT Ala	ej y	TCA Ser 255	777
TAT Tyr	TCC Ser	AAT Asn	TGG Trp	TTC Phe 260	TGG Trp	TAC Tyr	TAT	TGG Trp	GTT Val 265	Pro	CTT	TCT Ser	TTC Phe	Phe 270	822
GGA Gly	TTG Leu	ATG Met	CTC Leu	GTC Val 275	ATT Ile	GTT Val	ACC Thr	TAT Tyr	TTG Leu 280	Gln	CAT His	GTC Val	GAT Asp	GAT Asp 285	867
GTC Val	GCT Ala	GAG Glu	GTG Val	TAC Tyr 290	Glu	GCT Ala	GAT Asp	GAA Glu	TGG Trp 295	Ser	·TTC Phe	GTC Val	CGT Arg	GGA Gly 300	912
CAA Gln	ACC Thr	CAA Gln	ACC Thr	ATC Ile 305	Asp	CGT Arg	TAC Tyr	TAT	GGA Gly 310	. re:	GGA Gly	TTC Let	GAC L Asp	ACA Thr 315	957
Thr	Met	His	His	.Ile 320	Thr	Asp	Gly	His	325	PT	9 111:	5 112	5 F11	c. TTC e Phe 330	1062
Asn	Lys	Ile	Pro	His 335	Tyr	His	Let	· Ile	340) 1 VT	a Tn	 I GT		T GTC y Val 345	1047
Lys	Lys	Val	Leu	Gl 1 350	Pro	Leu	·Ser	Asp	35.	r G1 5	пту	I GI	, , , ,	C AAA T Lys 360	1092
Ser	Gln	Val	Asn	365	Asp) Phe	Phe	e A16	37	g Pn 0.	e re	:u 11	.p	C AAC ne Asn 375	1137
Туг	Lys	Leu	Asp	380	Let	ı Val	L Hi:	s Ly	s 10 - 38	5 A.	.a G.	LY I		IG CAA et Gln 390	i
TTC Phe	CGA Arg	ACA Thi	ACI Thr	Lei	GAC	a Gl	s aa u Ly	G GC	A AA a Ly 40	/S A.	la L	PG T. YS	AA		1221
AAGAATATCC CGTGCCGTTC TAGAGTACAA CAACAACTTC TGCGTTTTCA							1271								
CCGGTTTTGC TCTAATTGCA ATTTTTC111 GITCIATATA 1411-1-1-1							1321								
CTTTTTAATT TTATTCTCTC TAAAAAACTI CTACTITICA CIGCOTT							1371								
TGCATAAAGC CATAACTCTT								1391							

Fig. 17B

Optimized Ide-+ ---

caa gtt tga ggt ATG gtc gct cat tcc AGC gaa ggg CtG tcc gcc acg gct ccg gtc acc qqc gqC gat gtG ctg gtG gat gcC cgt gca tct ctG gaG gaG aag gag gcC ccC cgC gaC 61. oto aat gca aac act aaa cag gcc acc act gaG gag cc cgc atc caG tta cc act gtg 121. gat gcc ttc cgC cgC gca att ccC gca cac tgC ttc gaG agG gaC ctc gtG aaa tca atc 181. agG tat Ctg gtg caG gac ttt gcg gca ctG aca att ctG tac ttt gcC ctt ccC gcC ttt 241. gag tac ttt ggC Ctg ttt ggt tac Ctg gtG tgg aac att ttt atg ggC gtt ttt ggC ttc 301. gcg Ctg ttc gtc gtt gga cac gaC tgt ctt caC ggC tca ttc tcC gat aat cag aat ctc 361. aat gat tte att gga cat ate gee tte AGC cea ete tte tet ec tae tte ec tgg cag 421. aaa agt cac aag ctG cac caC gcC ttc acc aac cac atC gac aaa gat cat gga cac gtg 481. tgg atA cag gat aag gat tgg gaa gca atg cc AGC tgg aaa aga tgg ttc aat cc att 541. cell ttc tct ggC tgg ctG aaa tgg ttc cell gtg tac act CtG ttc ggt ttc tgC gat gga 601. tec cac ttc tgg cell tac tec tca cts ttt gts cgc aac tct gaa cgc gtt cas tgt gta 661. atc tot gga atc tgC tgc tgt gtg tgC gca tat att gct cta aca att gct gga AGC tat 721. too aat tog tto tog tac tat tog ott coa ott tot tto tto tgo tto atg otc otc att 781. att acc tat Ctg caC caC gtc gaC gtc gct gag gtg tac gag gct gat gaa tgg agc 841. tto gto cgG gga caG acc caG acc atc gat cgt tac tat ggC cto ggC ttg gac aca acg 901. atg cac cat atc aca gac gga cac gtt gcc cac ttc ttc aac aaa atc cca cat tac 961. 1021. cat ctc atc gaa gca acc gaa ggt gtc aaa aag gtc ttg gag ccg ttg tcc gac acc caa 1081. tac ggg tac aaa tct caG gtg aac tac gat ttc ttt gcc cgG ttc ctg tgg ttc aac tac 1141. aag ctc gac tat ctc gtt cac aag acc gcc gga atc atg caa ttc cga aca act ctc gag gag aag gca aag gcc aag tGa aag aat atc ccg tgc cgt tct aga gta caa caa cat 1201. ctg cgt ttt cac cgg ttt tgc tct aat tgc aat ttt tct ttg ttc tat ata tat ttt ttt 1261. 1321. gct ttt taa ttt tat tct ctc taa aaa act tct act ttt cag tgc gtt gaa tgc ata aag cca taa ctc tt

Fig. 18

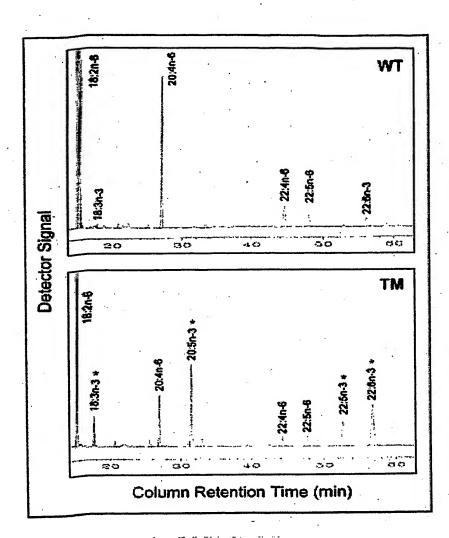


Fig. 19

